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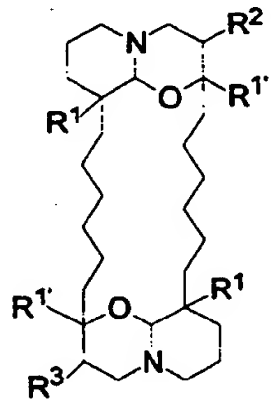
(54) Title: BIS-1-OXAQUINOLIZIDINE ALKALOIDS FROM A MARINE SPONGE WITH ANTITUMOR ACTIVITY

(57) **Abstract**

Compounds of general formula (II), or a pharmaceutically acceptable salt thereof, are of use as antitumor agents, where R^1 and $R^{1'}$ are the same or different, and each group is -H or -OH; the group R^2 is -H or $-CH_3$; and the group R^3 is -H or $-CH_3$; and can be isolated from marine sponges.



(II)

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Bis-1-oxaquinolizidine alkaloids from a marine sponge with antitumor activity

The present invention relates to *bis*-1-oxaquinolizidine alkaloids from a marine sponge. Such compounds have antitumor activity.

Background of the invention

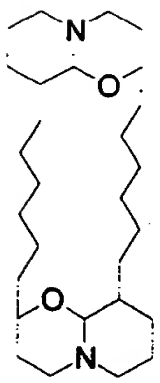
Recently, a number of complex macrocyclic alkaloids have been discovered from marine sponges which are known to process vasodilative activity. For example, quinolizidine alkaloids known as xestospongins and araguspongines have been isolated from sponges of the genus *Xestospongia* [Tet. Lett., 25, 3227, 1984; Tet. Lett. 30, 4149, 1989; and Chem. Pharm. Bull. 37, 1667, 1989].

Synthetic routes to some of these compounds and their precursors have also been published [Tet. Lett 31, 4281, 1990; Tet. Lett. 33, 507, 1992]. For example, (+)-*Xestospongin A* / (+)-*Araguspongine D* has been has recently been synthesised in its homochiral form [JACS, 116, 2617, 1994]. Also, other synthetic routes to these class of compounds have been proposed [Tet. Lett, 30, 4149, 1989].

Summary of the Invention

We have now found antitumor activity in such quinolizidine alkaloids and in structurally related novel quinolizidine alkaloids.

The present invention provides antitumor compounds with a core bis-1-oxaquinolizidine structure found in the known compounds. The core bis-1-oxaquinolizidine structure is of formula (I), as follows:



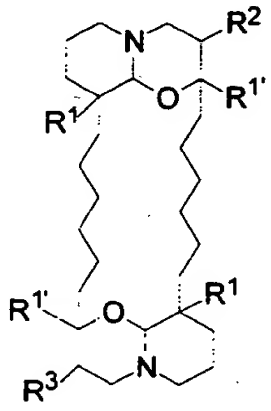
(I)

Thus. the invention also provides antitumor compositions and methods using an antitumor compound incorporating the core structure of formula (I).

The compounds of this invention have optical centres, and the present invention extends to the individual isomers as well as mixtures including the racemic compounds.

Preferred embodiments of the invention

More particularly, the antitumor compounds of the present invention are of the formula (II):



(II)

where groups R^1 and $R^{1'}$ are the same or different and each group is -H or -OH. the group R^2 is -H or $-CH_3$, and the group R^3 is -H or $-CH_3$. Demethylxestospongin B and Araguspongine A. C and D are known compounds within this general formula.

The compounds of this invention are identified as compounds in the series "LT", and structures for individual LT compounds are given later in this description.

Preferably at least two of the groups R^1 are -OH. More preferably, the antitumor compound is one of the known compounds, especially araguspongine C (LT-1), or one of the new compounds LT -5 to LT-10.

The compounds of the invention can be easily transformed into physiologically acceptable salts by methods known in the art. Such salts include acid addition salts formed with inorganic or organic acids, such as HBr, HCl, H_2SO_4 or HOAc.

The compounds LT-1 and LT-5 show promising antitumor activity, indicating similar activity for other compounds with the core bis-1-oxaquniolizine structure of formula (II).

Examples of pharmaceutical compositions provided by this invention include solid (tablets, pills, capsules, granules, etc.) or liquid (solutions, suspensions or emulsions) formulations with a suitable composition for oral, topical or parenteral administration. They may contain the pure compound or in combination with any other pharmacologically active compound. These compositions may need to be sterile when administered parentally.

The correct dosage of a pharmaceutical composition comprising a compound of formula (II) will vary according to the pharmaceutical formulation, the mode of application, and the particular situs. host and tumor being treated. Other factors like age, body weight sex, diet, time of administration, rate of excretion, condition of the host, drug combinations, reaction sensitivities and severity of the disease shall be taken into

account. Administration can be carried out continuously or periodically within the maximum tolerated dose.

The marine sponge is of the genus *Xestospongia* sp. and was obtained in the Bay of Thailand and also at Kohama Island in Okinawa [which is very close to Aragusuku Island, which is the site of collection of the sponge discussed in Tet. Lett. 30. 4149. 1989; and Chem. Pharm. Bull. 37, 1667, 1989]. A specimen of the sponge has been deposited at Chulalongkorn University, Bangkok, Thailand (deposit number PH-5-94-008). The alkaloids isolated from this sponge have been found to have antitumor activity.

Compounds of the general formula (II) can be made by synthetic or semi-synthetic routes[Tet. Lett 31, 4281, 1990; Tet. Lett. 33, 507, 1992]. For example, (+)-*Xestospongin A* / (+)-*Araguspongine D* has been has recently been synthesised in its homochiral form [JACS, 116, 2617, 1994]. Also, other synthetic routes to these class of compounds have been proposed [Tet. Lett, 30, 4149, 1989].

From the methodology recently published in the literature, the compounds of this invention can easily be synthesised. For example, the work carried out by T. Hoye [JACS, 116, 2617, 1994] shows the total synthesis of *Araguspongine D*, which is structurally very similar to the compounds claimed in the invention. Using this synthetic route and starting off with the appropriate chiral or racemic amino alcohol, having a methyl substituent at the β-position, leads to the compound of the formula LT-8 of the present invention. With the compound LT-8 in hand, other derivatives in the invention can be made by regio and stereoselective oxidation at the C-9 position on the oxaquinolizidine ring. The separation of stereoisomers generated in the reaction can be separated by known methods. Conversion of one compound to another and derivatisation of isolated quinolizidines, is also feasible.

Examples of the invention

The present invention is illustrated by the following examples, which also includes details for isolation of some of the known compounds.

Isolation of five Macrocyclic Alkaloids from an Unidentified Marine Sponge

A fresh sample of a sponge belonging to the genome *Xestospongia* sp. (wet 77g) [deposited at Chulalongkorn University, Bangkok, Thailand, deposit number PH-5-94-008] was collected by hand using SCUBA at sandy-rocky bottom (7m) of Coral Island near Phuket. Thailand. This species belonged to the class Demospongiae and resembles the Okinawan sponge *Xestospongia* sp. which yielded similar compounds. The sponge sample was extracted with acetone (3L) three times. After filtration and concentration. the resulting aqueous residue was partitioned between EtOAc and water. MeOH was added to the water layer, filtered and concentrated to give MeOH soluble extract of 1.3g.

Gross separation of the MeOH soluble extract was carried out by vacuum flash chromatography (silica, 200g) using mixtures of CH_2C1_2 and MeOH into ten fractions The steps involved in the separation are shown in Figure 1 of the accompanying drawings. The ninth fraction (130mg) was separated by open column chromatography to give four subfractions using EtOAc and MeOH mixtures. The second subfraction (30.0mg) was further separated by repeated HPLC on reversed-phase column (RP-18) using MeOH to furnish five pure alkaloids. Similar compounds were obtained from fraction 10 (from VFC) and these were combined with LT-1 (10mg), LT-2 (5.5mg), LT-3 (4.3MG), LT-4 (3.1mg), LT-5 (1.5mg) and LT-6 (2.0mg).

Based on 1D NMR spectral data the first four metabolites are known compounds; Araguspongine C, Araguspongine A, Demethylxestospongine B and Araguspongine D respectively. Compounds LT-5 and LT-6 are new.

Four more additional new macrocyclic alkaloids were isolated from fraction 5 and 6 respectively by HPLC on reversed-phase column (RP-18) using MeOH. These were designated as LT-7 (2mg), LT-8 (1mg), LT-9 (1.2mg) and LT-10 (1.4mg).
The compounds isolated were characterised by spectroscopic and analytical methods and their data is shown below:

Araguspongine A: white crystals, mp 155-156°C; $[\alpha]_D^{26}$+17.1°(c = 1.0, $CHCl_3$); IR ($CHCl_3$) 3500, 2920, 2855, 2800, 2750, 1460, 1125, 1086 cm^{-1}; MS *m/z* 462 (M^+, 23). 69 (100); HREIMS obsd 478.3771, calcd for $C_{28}H_{50}N_2O_4$ 478.3768.

Araguspongine C: white crystals, mp 148-150°C $[\alpha]_D^{26}$ + 11° (c = 0.7, $CHCl_3$); IR ($CHCl_3$), 3500, 2930, 2865, 1462, 1132, 1096, 1020 cm^{-1}; MS *m/z* 478 (M^+, 11); HREIMS obsd. 478.3771, cald for $C_{28}H_{50}N_2O_4$ 478.3768.

Araguspongine D: amorphous; $[\alpha]_D^{26}$ -5.1° (c = 0.5, $CHCl_3$); IR ($CHCl_3$) 2940, 2860, 2810, 2760, 1125, 1090 CM^{-1}; ms *M/Z* 446 (M^+, 33); HREIMS obsd. 466.3889, calcd for $C_{28}H_{50}N_2O_2$ 446.3870.

Demethylxestospongine B: amorphous; $[\alpha]_D^{20}$+6° (c = 0.8, $CHCl_3$); IR ($CHCl_3$) 3450, 2931, 1520, 1500 cm^{-1}; MS *m/z* 462 (M^+, 100); HREIMS obsd. 462.3833, calcd for $C_{28}H_{50}N_2O_3$ 462.3818.

Compound LT-5: amorphous; IR ($CHCl_3$) 3500, 2910, 2845, 2750, 1465, 1134 cm^{-1} MS *m/z* 476 (M^+·100), HREIMS obsd 476.3969, calcd. for $C_{29}H_{52}N_2O_3$ 476.3974.

Compound LT-6: amorphous; IR ($CHCl_3$) 3500, 2910, 2845, 1460, 1095, 1015 cm^{-1} MS *m/z* 492 (M^+, 100); HREIMS obsd. 492.3915, Calcd. for $C_{29}H_{52}N_2O_4$ 492.3924.

Compound LT-7: amorphous; IR ($CHCl_3$) 3500, 2910, 2845, 1130, 1015 cm^{-1};MS *m/z* 490 (M^+, 100); HREIMS obsd. 490.4113, calcd. for $C_{30}H_{54}N_2O_3$ 490.4131.

Compound LT-8: amorphous: IR ($CHCl_3$) 2910, 2860, 2810, 1460, 1095, 1015 cm^{-1} MS *m/z* 474 ($M^{+\cdot}$ 100); HREIMS obsd. 474.4173, calcd for $C_{30}H_{54}N_2O_2$ 474.4182.

Compound LT-9: amorphous; IR ($CHCl_3$), 3500, 2930, 2865, 1460, 1135, 1100 cm^{-1};MS *m/z* 506 ($M^{+\cdot}$ 100); HREIMS obsd. 506.4081, calcd for $C_{30}H_{54}N_2O_4$ 506.4081.

Compound LT-10: amorphous, IR ($CHCl_3$) 3550, 2950, 2850, 1460, 1430, 975 cm^{-1}; EI *m/z* 490(M^{+}); HREIMS obsd 490.4149, calcd for $C_{28}H_{50}N_2O_4$ 490.4131.

Table 2. ^{1}H and ^{13}C NMR data of araguspongine C, LT-6 and LT-5 (in $CDCl_3$).

	AragusponginеC (LT-1)		LT-6		LT-5	
No	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
2	76.5d	3.56 brt	76.5 d	3.59 brt	82.5 d	3.09 dd
4	52.6 t	3.11 brt 2.97 brdd	52.2t	3.22 brt 3.03 brdd	60.4 t	2.85 dd 2.63 brt
6	44.3 t	3.03 ddd 2.34 brd	44.3 t	3.09 m 2.47 brd	45.2 t	2.39 brdd 3.0 m
9	70.8 s	-	70.7 s	-	70.7	-
10	90.4 d	4.06 s	90.4 d	4.16 s	90.3 d	4.03 s
3Me					14.6 q	0.69 d
2'			82.7	3.15 ddd	75.2 d	3.35 brt
4'			59.7 t	2.91 brdd 2.74 brt	54.1t	2.17 ddd 2.94 m
6'			45.2 t	2.52 brd 3.05 m	54.3 t	1.98 ddd 2.76 brd
9'			70.7 s	-	40.3 d	-
10'			90.2 d	4.12 s	95.5 s	3.10 d
3'Me			14.5 q	0.72d		
	38.6 t		38.7 t		38.6 t	
	36.4 t		38.6 t		35.5 t	
	32.4 t		35.9 t		32.7 t	
	31.6 t		32.8 t		32.3 t	
	29.7 t		31.9 t		32.2 t	
	26.1 t		31.9 t		31.7 t	
	25.0 t		31.4 t		31.7 t	
	22.6 t		31.4 t		31.6 t	
	21.0 t		29.7 t		31.2 t	
			29.4 t		29.4 t	
			28.3 t		29.2 t	

			25.7 t		28.8 t	
			24.8 t		25.4 t	
			24.8 t		24.9 t	
			22.5 t		24.9 t	
			22.5 t		24.8 t	
			20.4 t		22.7	
			20.4 t		20.9 t	

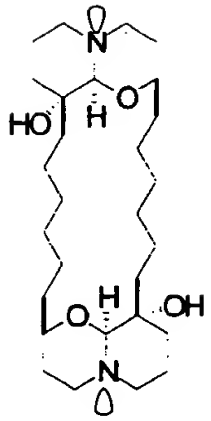
Table 3. ^{1}H and ^{13}C NMR data of LT-7, LT-8 and LT-9 (in $CDCl_3$)

	LT-7		LT-8		LT-9	
No.	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
2	82.4d	3.12d	81.0d	2.95	82.7	3.17 brt
4	60.6t	2.85dd 2.63 brt	62.2t	1.80m 2.83 brt	59.3	2.93 brdd 2.81 brt
6	45.9t	3.04 brt	53.8	1.97ddd 2.75	45.2t	3.09brt 2.57brd
9	70.7s	-	40.5 d	-	70.8g	-
10	90.1d	4.03s	95.7d	3.04m	90.2d	4.18s
3Me	14.5q	0.67d	14.8q	0.75d	14.4q	
2'	81.9d	3.09d	81.8d	3.09brt		
4'	60.3t	2.83dd 2.71brt	60.6t	2.86m 2.74m		
6'	45.1t	2.99brt 2.39brd	45.1t	3.04m 2.47brd		
9'	40.3d		40.5d			
10'	87.1d	4.27brd	87.3d	4.28		
3'Me	14.5q	0.67	14.6q	0.67d		
	38.6t		33.1t		38.8t	
	33.2t		32.6t		32.7t	
	32.8t		32.5t		31.7t	
	32.2t		31.8t		31.4t	
	31.8t		31.8t		29.6t	
	31.7t		31.3t		28.4t	
	29.7t		29.8t		24.8t	
	29.6t		29.5t		22.6t	
	28.3t		29.0t		20.2t	
	28.2t		28.8t			

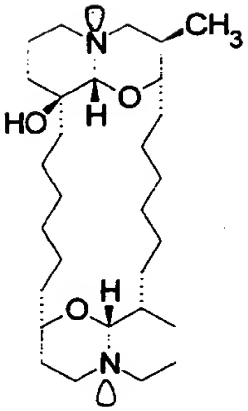
	27.4t		28.5t			
	26.4t		27.4t			
	25.7t		26.4t			
	24.8t		25.7t			
	24.8t		25.3t			
	22.8		25.1t			
	20.9t		24.9t			
			24.7t			

Table 4. ^{1}H and ^{13}C NMR data of LT-10 (in $CDCl_3$)

LT-10	
^{13}C	^{1}H
95.3 d	4.07s
90.3 d	3.12rbt
82.4d	3.02m
81.3d	3.02 brt
70.7s	2.88brdd
62.2t	2.69 brt
59.9t	2.46
53.7t	0.79 d
45.2	0.70 d
39.6d	
38.6t	
35.1t	
32.1t	
31.9t	
31.6t	
31.5t	
30.9t	
29.5t	
28.3t	
24.9t	
24.8t	
24.6t	
22.7t	
20.6t	
14.5q	
14.4q	



Araguspongine C



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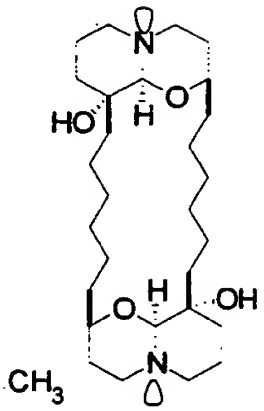

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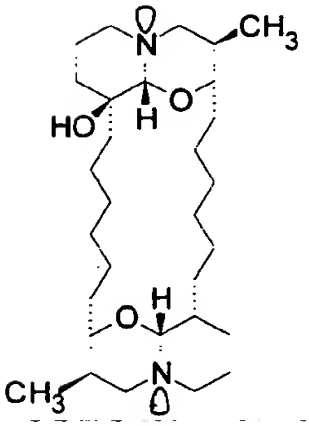

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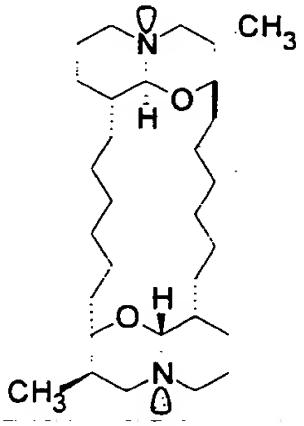

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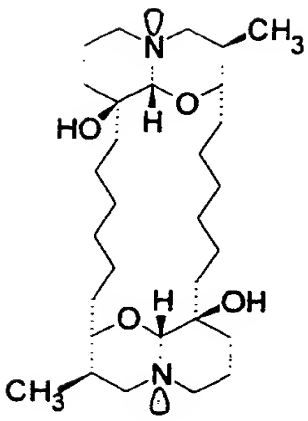

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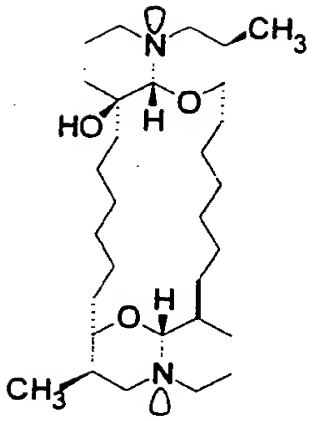


LT-10

Biological Activity

Cell Cultures

Cells were maintained in logarithmic phase of growth in Eagle's Minimum Essential Medium, with Earle's Balanced Salts, with 2.0 mM L-glutamine, with non-essential amino acids, without sodium bicarbonate (EMEM/neaa); supplemented with 10% Fetal Calf Serum (FCS), 10^{-2} M sodium bicarbonate and 0,1 g/l penicillin-G + streptomycin sulphate.

A simple screening procedure was carried out to determine and compare the antitumor activity of these compounds, using an adapted form of a literature method. The antitumor cells employed were P-388 (suspension culture of a lymphoid neoplasm from DBA/2 mouse), A-549 (monolayer culture of a human lung carcinoma), HT-29 (monolayer culture of a human colon carcinoma) and MEL-28 (monolayer culture of a human melanoma).

P-388 were seeded into 16 mm wells at 1 x 10^4 cells per well in 1 ml aliquots of MEM 5FCS containing the indicated concentration of drug. A separate set of cultures without drug were seeded as control growth to ensure that these cells remained in exponential phase of growth. All determinations were carried out in duplicate. After three days of incubation at 37°C, 10% CO_2 in an atmosphere of 98% humidity, the wells were stained with 0.1% Crystal Violet. An approximate IC_{50} was determined by comparing the growth in wells with drug to the growth in wells control.

A-549, HT-29 and MEL-28 cells were seeded into 16 mm wells at 2 x 10^4 cells per well in 1 ml aliquots of MEM 10FCS containing the indicated concentration of drug. A separate set of cultures without drug was seeded as control growth to ensure that cells remained in exponential phase of growth. All determinations were carried out in duplicate. After three days of incubation at 37°C, 10% CO_2 in an atmosphere of 98% humidity, the wells were stained with 0.1% Crystal Violet. An approximate IC_{50} was determined by comparing the growth in wells with drug to the growth in wells control.

Results:

	$IC_{50}\mu g/ml$			
COMPOUNDS	P-388	A-549	HT-29	MEL 28
LT-1	0.01	0.01	0.025	0.025
LT-5	0.1	0.1	0.1	0.25
LT-6	0.1	0.1	0.25	0.25
T-7	0.12	0.12	0.12	0.25
LT-8	>1	>1	>1	>1
LT-9	0.5	0.5	0.5	1
LT-10	1	0.5	0.5	1

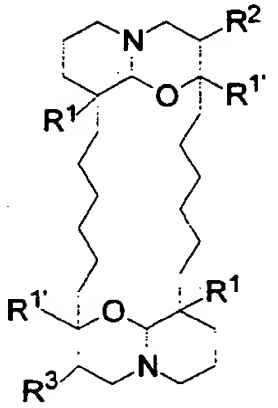
Literature References

Biochem. Bioph. Res. Comm. 1984, 3, 848-854.

J. Med. Chem 1981, 24, 1078-1083.

CLAIMS:

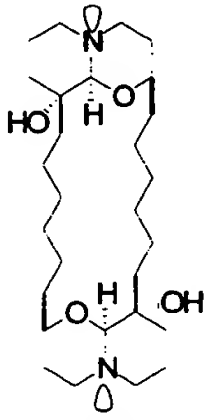
1. The use of a compound of the general formula II, or a pharmaceutically acceptable salt thereof,



(II)

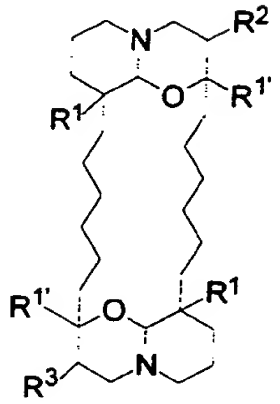
where R^1 and $R^{1'}$ are the same or different, and each group is -H or -OH;
the group R^2 is -H or $-CH_3$;
and the group R^3 is -H or $-CH_3$;
in the preparation of a medicament for use as an antitumor agent.

2. The use according to Caim 1 wherein the compound of the general formula II is a compound LT-1 of the formula III



(III)

3. A compound of the general formula II:



(II)

or a pharmaceutically acceptable salt thereof,

where R^1 and $R^{1'}$ are the same or different, and each group is -H or -OH;

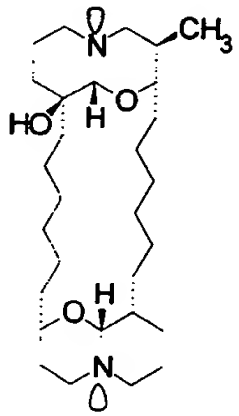
the group R^2 is -H or $-CH_3$;

and the group R^3 is -H or $-CH_3$;

with the exclusion of Demethylxestospongin B, Araguspongine A, Araguspongine C and Araguspongine D.

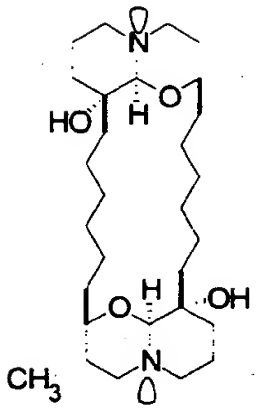
4. A compound according to Claim 3 selected from:

LT-5 of the formula (IV);



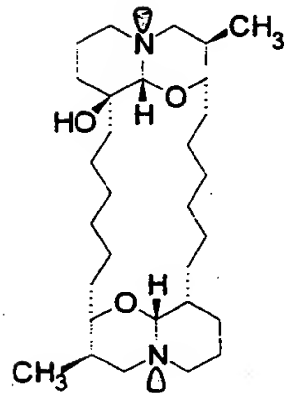
(IV)

LT-6 of the formula (V);



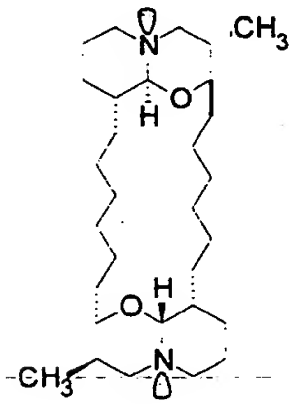
(V)

LT-7 of the formula (VI);



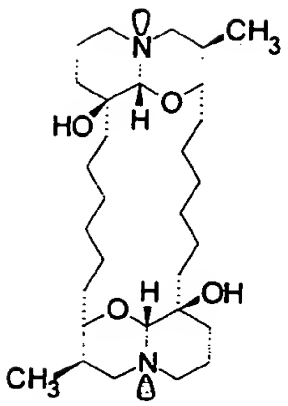
(VI)

LT-8 of the formula (VII);



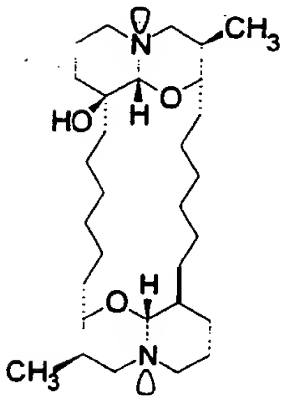
(VII)

LT-9 of the formula (VIII);



(VIII)

or LT-10 of the formula (IX).

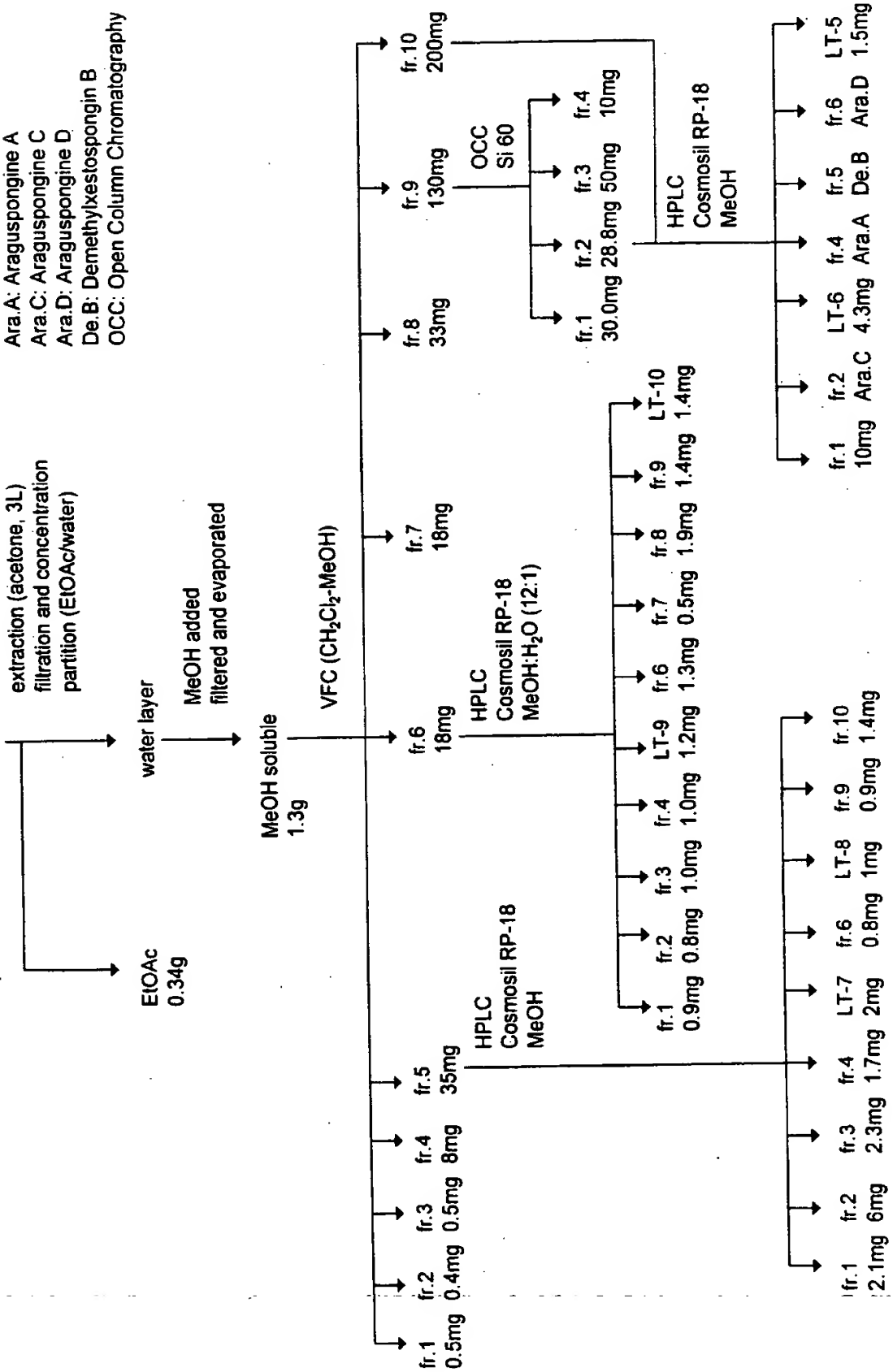


(IX)

5. A compound as claimed in Claim 3 or 4, for use as an antitumor agent.

6. A pharmaceutical composition containing a compound as claimed in Claims 3, 4 or 5 and a pharmaceutically acceptable carrier.

Figure 1


Xestospongia sp. (77g, wet)
extraction (acetone, 3L)
filtration and concentration
partition (EtOAc/water)
EtOAc
0.34g
water layer
MeOH added
filtered and evaporated
MeOH soluble
1.3g
VFC (CH_2Cl_2-MeOH)
fr.1 0.5mg
fr.2 0.4mg
fr.3 0.5mg
fr.4 8mg
fr.5 35mg
HPLC
Cosmosil RP-18
MeOH
fr.6 18mg
HPLC
Cosmosil RP-18
MeOH:H_2O (12:1)
fr.7 18mg
fr.8 33mg
fr.9 130mg
fr.10 200mg
OCC
Si 60
fr.1 0.9mg
fr.2 0.8mg
fr.3 1.0mg
fr.4 1.0mg
LT-9 1.2mg
fr.6 1.3mg
fr.7 0.5mg
fr.8 1.9mg
fr.9 1.4mg
LT-10 1.4mg
fr.1 30.0mg
fr.2 28.8mg
fr.3 50mg
fr.4 10mg
HPLC
Cosmosil RP-18
MeOH
fr.1 2.1mg
fr.2 6mg
fr.3 2.3mg
fr.4 1.7mg
LT-7 2mg
fr.6 0.8mg
LT-8 1mg
fr.9 0.9mg
fr.10 1.4mg
fr.1 10mg
fr.2 Ara.C
LT-6 4.3mg
fr.4 Ara.A
fr.5 De.B
fr.6 Ara.D
LT-5 1.5mg
Ara.A: Araguspongine A
Ara.C: Aragusponginе C
Ara.D: Aragusponginе D
De.B: Demethylxestospongin B
OCC: Open Column Chromatography

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(71) Applicant *(for all designated States except AT AU BE BR CA CH DE DK ES FI FR GB GR IE IT JP KR LU MC MX NL NZ PT RU SE UA US)*: RUFFLES, Graham, Keith [GB/GB]; 57-60 Lincoln's Inn Fields, London WC2A 3LS (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: TANAKA, Jun-ichi [JP/JP]; 18-307, 1386 Maeda, Urasoe, Okinawa 901-21 (JP). HIGA, Tatsuo [JP/JP]; 7-202, 1-62-3 Shuri Ishimine, Naha, Okinawa 903 (JP). GARCIA GRAVALOS, Dolores [ES/ES]; Calle Maldonado, 63, E-28006 Madrid (ES).

(74) Agent: RUFFLES, Graham, Keith; Marks & Clerk, 57-60 Lincoln's Inn Fields, London WC2A 3LS (GB).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

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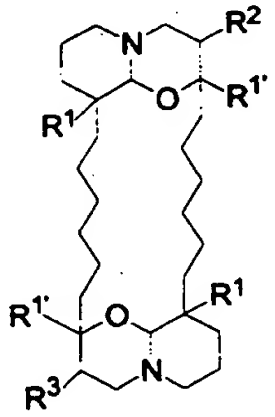
(57) Abstract

Compounds of general formula (II), or a pharmaceutically acceptable salt thereof, are of use as antitumor agents, where R^1 and $R^{1'}$ are the same or different, and each group is -H or -OH; the group R^2 is -H or $-CH_3$; and the group R^3 is -H or $-CH_3$; and can be isolated from marine sponges.



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INTERNATIONAL SEARCH REPORT

Interna J Application No
PCT/GB 96/01852

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/535

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	PLANTA MEDICA, vol. 62, no. 1, February 1996, pages 28-30, XP000617775 VASSAS, A. ET AL: "Naturally occurring somatostatin and vasoactive intestinal peptide inhibitors. Isolation of alkaloids from two marine sponges." see the whole document ---	1,3,5,6
P,X	BIOORGANIC AND MEDICINAL CHEMISTRY LETTERS, vol. 6, no. 12, 1996, pages 1313-1318, XP000617820 PETTIT, G.R. ET AL: "Isolation and X-ray crystal structure of racemic Xestospongin D from the singapore marine sponge Niphates sp." see the whole document ---	1,6

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[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
20 February 1997	17.03.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Mair, J

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF NATURAL PRODUCTS, vol. 55, no. 10, 1992, pages 1505-1508, XP000617818 QUIRION, J.-C. ET AL: "Two new alkaloids from Xestospongia sp., a New Caledonian sponge" see the whole document especially compound 2 and table 3 ---	1,3,5,6
X	CHEM. PHARM. BULL., vol. 37, no. 6, 1989, pages 1676-1678, XP000617987 KOBAYASHI, M. ET AL: "Araguspongines B, C, D, E, F, G, H, and J. New vasodilative bis-1-oxaquinolizidine alkaloids from an Okinawan marine sponge, Xestospongia sp." see the whole document ---	3
X	CANCER CHEMOTHERAPY AND PHARMACOLOGY, vol. 30, no. 5, 1992, pages 401-406, XP000617821 STINGL, J. ET AL: "In vitro screening of crude extracts and pure metabolites obtained from marine invertebrates for the treatment of breast cancer" see the whole document ---	1,3,5,6
X	JOURNAL OF NATURAL PRODUCTS, vol. 57, no. 9, 1994, pages 1283-1285, XP000617823 VENKATESWARLU, Y. ET AL: "bis-1-Oxaquinolizidines from the sponge Haliclona exigua" see page 1283, left-hand column, line 21 - right-hand column, line 7 & compound 1 ---	3,4,6
X	DATABASE WPI Week 8506 Derwent Publications Ltd., London, GB; AN 85-034355 XP002025798 & JP 59 227 885 A (SUMITOMO RUBBER IND KK) , 9 June 1983 see abstract --- -/--	3,6

1

INTERNATIONAL SEARCH REPORT

Interna l Application No
PCT/GB 96/01852

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF ORGANIC CHEMISTRY, vol. 59, no. 23, 1994, pages 6904-6910, XP000644369 HOYE, T.R. ET AL: "Conformational considerations in 1-oxaquinolizidines related to the xestospongin/araguspongine family: Reassignment of stereostructures for araguspongines B and E" see the whole document & JOURNAL OF ORGANIC CHEMISTRY, vol. 60, no. 15, 1995, page 4958 HOYE, T.R. ET AL: "Additions and corrections" see the whole document ---	3
X	TETRAHEDRON LETTERS, vol. 30, no. 31, 1989, pages 4149-4152, XP000615876 KOBAYASHI, M. ET AL: "Aragopetrosine A, a new vasodilative macrocyclic quinolizidine alkaloid from an Okinawan marine sponge Xestospongia sp." cited in the application see compounds 2 and 8 ---	3
X	TETRAHEDRON LETTERS, vol. 25, no. 30, 1984, pages 3227-3230, XP000615875 NAKAGAWA, M. ET AL: "Structures of xestospongin A,B,C and D novel vasodilative compounds from marine sponge, Xestospongia exigua" cited in the application see compounds 1-4 -----	3